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Soil enzyme activities and physical properties in a watershed managed under agroforestry and row-crop systems

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ABSTRACT

The proportion of water-stable aggregates (WSA) and a diverse microbial activity influence soil quality, crop growth, nutrient retention, water infiltration, and surface runoff. The objective of the study was to test the hypothesis that permanent vegetative buffers increase WSA and contribute to increased soil enzyme activity. Soil samples (5 cm diameter and 10 cm long) from agroforestry (AG), grass buffer (GB), grass waterway (GWW) and crop (CS) areas were collected from summit, middle, and lower landscape positions at the Paired Watershed Study, near Novelty, MO. Water-stable aggregates (>250 μm diameter; wet-sieving method), soil carbon, and soil enzyme activity were determined and data were statistically analyzed. Soils under permanent vegetative buffers and GWW had significantly lower bulk density and more WSA than the crop areas. Soil carbon contents were highest in the GWW and lowest in the CS treatments. Fluorescein diacetate (FDA) hydrolase, β-glucosidase, and glucosaminadase enzyme activities were higher in AG, GB, and GWW soils than CS soils. Dehydrogenase activity differed between grass buffer or GWW and crop areas. The results of the study show that WSA, soil carbon, and functional diversity of enzyme activity increased due to establishment of buffers with trees and grass. It can be speculated that increased diversity of functional enzymes associated with cycling of key soil nutrients and improved soil physical properties may reduce nonpoint source pollution (NPSP) from row-crop agriculture watersheds thus improving overall environmental quality.

1. Introduction

Agroforestry practices are considered to be environmentally friendly, help diversify farm income, and can play a major role in the mitigation of green house gases and the adaptation of agriculture to changing environmental conditions (Gold and Hanover, 1987; Garrity, 2004). Research in the temperate zone has shown that agroforestry practices improve water quality and certain soil physical properties, and increase carbon sequestration (Udawatta et al., 2002; Montagnini and Nair, 2004; Mungai et al., 2005; Seobi et al., 2005). It can be speculated that these environmental benefits also are associated with soil microbial activity and soil biological parameters. For example, mineralization of herbicides has been observed in surface and subsurface soils (Larsen et al., 2000; Wood et al., 2002).

Many soil biological processes are carried out by soil microorganisms (Sørensen and Sessitsch, 2007). Soil microbial communities are attached to internal and external surfaces of soil aggregates and pore spaces and therefore, persistence of aggregates are important for biogeochemical soil processes (Park and Smucker, 2005; Standing and Killham, 2007). The percentage of water-stable aggregates (WSA) is a measure of resistance to breakdown by water and mechanical manipulation. Macroaggregates (diameter >250 μm) are considered as a secondary soil structure associated with pores, microbial habitat, and physical protection of organic matter (Christensen, 2001; Carter, 2004). Immediately after cultivation, most soils contain large pores which are important for water and gas movement and their continued existence is determined by aggregate stability (Kemper and Rosenau, 1986). According to Six et al. (2006) soil microbes improve soil aggregation and therefore, WSA may be used as an indirect measure of enzyme activity.

Enzyme assays provide quantitative information on functional diversity of microbial activity, soil chemical processes, mineralization rates, and organic matter accumulation. Some enzymes are routinely produced by microbial cells while others are formed

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in the presence of an appropriate substrate (Kandeler, 2007). Enzyme assays among different management practices may also indicate short-term differences in soil quality improvement, functional diversity of critical soil processes, rapid responses to changes in management, and sensitivity to environmental stresses (Dick, 1997; Nannipieri et al., 2002; Caldwell, 2005). Studies show that enzyme activity and microbial diversity are greater in agroforestry alley cropping practices due to differences in litter quality and quantity, and root exudates (Gomez et al., 2000; Myers et al., 2001; Mungai et al., 2005; Sørensen and Sessitsch, 2007). The presence of a large and diverse soil microbial community is crucial to the productivity of any agroecosystem. Previous research suggests that relationships between organic matter, microbial activity, and microbial biomass are good indicators of soil maturity (Anderson and Domsch, 1990; Insam and Domsch, 1988).

Fluorescein diacetate (FDA) hydrolase, β-glucosidase, glucosaminadase, and dehydrogenase activities represent a broad spectrum of enzymatic activities in soils. These enzymes are involved with decomposition of complex organic compounds, and nitrogen mineralization, and are correlated with fungal and microbial biomass (Stevenson, 1959; Sinsabaugh and Moorhead, 1995; Dick et al., 1996; Miller et al., 1998; Gasper et al., 2001; Kandeler, 2007).

Although several environmental benefits of agroforestry practices are reported in the literature, more research is needed to fill key knowledge gaps for a comprehensive understanding of buffer effects on overall environmental quality (Lowrance et al., 2002; Loveall and Sullivan, 2006). Studies of buffer influence on WSA, soil microbial functional diversity, and soil enzyme activity are limited in the literature. A better understanding of microbial activity is also important for management of carbon and nitrogen stocks in soils (Allison et al., 2005). We hypothesized that adoption of grass and agroforestry buffer practices would improve soil properties and soil enzyme activity. The objective of this study was to compare differences in water-stable aggregates, soil carbon, and enzyme activities in crop, grass buffer, agroforestry buffer, and grass waterway areas at three landscape positions in agroforestry and grass buffer alley cropping watersheds.

2. Materials and methods

2.1. Watershed description

The two study watersheds are located in Knox County, Missouri, USA (40°01′N, 92°11′W; Fig. 1). Since 1991, watersheds were under a corn (*Zea mays* L.)–soybean (*Glycine max* (L.) Merr.) rotation with no-till land preparation. Grass buffers (3–4.5 m wide) consisting of redtop (*Agrostis gigantea* Roth), brome grass (*Bromus* spp.), and birdsfoot trefoil (*Lotus corniculatus* L.) were established 36.5 m apart on both watersheds in 1997. Pin oak (*Quercus palustris* Muenchh.) trees were planted in the center of the buffer strips at 10-m spacing in the agroforestry watershed. The two watersheds were identified as grass buffer (3.16 ha) and agroforestry buffer (4.44 ha). Grass waterways on both watersheds consist of Kentucky 31 tall fescue (*Festuca arundinacea* var. *genuina* Schreb.).

Watersheds are underlain by glacial till and Peorian loess material (Unklesbay and Vineyard, 1992). Soils in the watersheds are Putnam silt loam (fine, smectitic, mesic Vertic Albaqualfs) and Kilwinning silt loam (fine, smectitic, mesic Vertic Epiaqualfs) with a minor proportion of Armstrong silt loam (fine, smectitic, mesic Aquertic Hapludalfs) on steeper, 5–9% slopes (Watson, 1979). The argillic horizon in these soils severely restricts vertical soil water percolation and copious surface runoff is produced during saturation periods in the spring and early summer, and large or closely spaced small precipitation events.

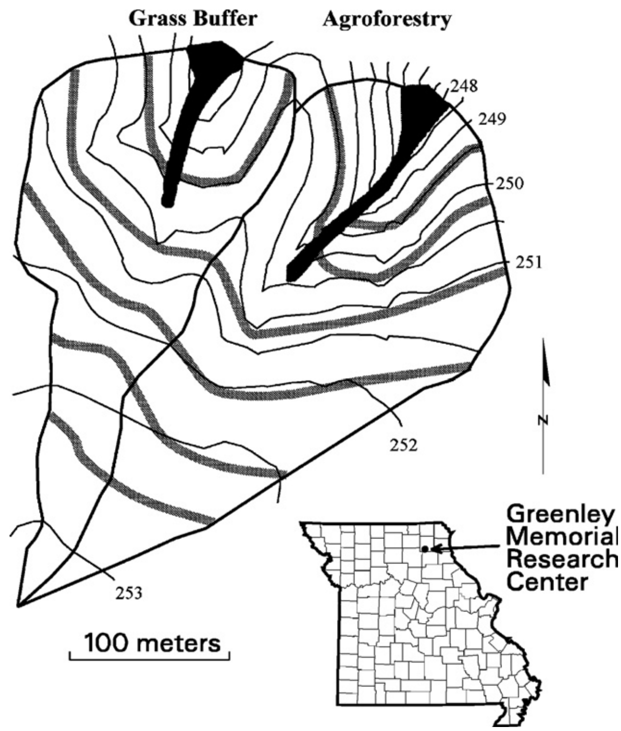

Fig. 1. Topographic map of the grass buffer and the agroforestry watersheds with 0.5 m elevation interval contour lines (black), grass (grass only), agroforestry (grass + trees) buffers (gray), and grass waterways (wide black). The inset map shows the location of watershed in Knox County, Missouri, USA.

About 66% (600 mm) of the 30-year mean annual precipitation in the region (920-mm) falls from April through September (Owenby and Ezell, 1992). Mean annual air temperature is approximately 11.7 °C, with an average monthly low of −6.6 °C in February and an average monthly high of 31.4 °C in July (Owenby and Ezell, 1992). Snowfall averages about 590 mm yr^{-1} and can stay on the ground for extended periods.

2.2. Analysis of samples

Treatments were grass buffer (GB), agroforestry buffer (AG), crop (CS), and grass waterway (GWW) areas. Two transects extending from the summit to the lower backslope landscape positions were identified on both watersheds in September 2006 to collect surface 0–10 cm soils. Samples for the AG and GB treatments were collected from the first, third, and fifth buffers (counting from the south; Fig. 1) and these buffers represented upper, middle, and lower landscape positions, respectively. Soil samples were collected from the center of the grass buffer and about 40 cm from the base of the tree trunk for the GB and AG treatments, respectively. Sites about 2-m south of the buffer edge in the crop area were sampled for the CS treatment. For the GWW treatment, soils were collected from three locations (south, middle, and north) within the grass waterway. Soils were sampled with a 5-cm diameter auger, placed in a labeled ziplock bag, and transported to the laboratory in a cooler. Samples were stored at 4 °C prior to measurements being conducted.

A method described by Kemper and Rosenau (1986) and modified by Angers and Mehuys (1993) was used to determine water-stable aggregates. Briefly, a 10-g soil sample with two replications was used to determine aggregates >250 μm diameter with the wet-sieving method. Total organic carbon concentration was determined by combustion analysis at 950 °C using a LECO TruSpec CN Analyzer. A previous sample set collected in 2002 from the same treatments was used to compare changes in carbon and to include soil bulk density values. Bulk density values from the Seobi et al. (2005) study were used for the 2006 sampling.

Standard methods were used to determine enzyme activity. The activity of β-glucosidase enzyme was expressed as μg *p*-nitrophenol released g^{-1} dry soil h^{-1} at 410 nm (Dick et al., 1996). Glucosaminadase enzyme activity was determined as described by Parham and Deng (2000) and the concentration of *p*-nitrophenol was measured colorimetrically (405 nm) and the enzymatic activity was expressed as μg *p*-nitrophenol released g^{-1} soil h^{-1}. Soil was incubated with 2,3,5-triphenyltetrazolium chloride substrate at 37 °C for 24 h and the concentration of the triphenyl formazan (TPF) product was colorimetrically (485 nm) measured and the dehydrogenase enzymatic activity was expressed as μg TPF released g^{-1} dry soil h^{-1} (Pepper et al., 1995). FDA activity was determined according to Dick et al. (1996) and expressed as μg fluorescein released g^{-1} dry soil h^{-1} at 490 nm. Since the effect of landscape position was not significant on enzyme activity, results for landscape effect were not presented. Similar findings have been observed in a soil enzyme study conducted in Ohio (Decker et al., 1999).

Statistical analyses of data were performed using Statistical Analysis Systems Software (SAS Institute, 1999). The data were analyzed according to a completely randomized design with four management levels and three landscape positions with two replications. Proc GLM and MIXED procedures in SAS were used to determine differences among treatments. Least squares regression analysis (Proc REG) was used to describe relationships between parameters. Differences between treatments and landscape positions were declared significant at the $\alpha = 0.05$ level. Computed tomography-measured soil parameters for the 0–10 cm depth for these treatments (Udawatta et al., 2006) were compared with measured soil enzyme activities to evaluate relationships between soil pore characteristics and enzyme activity among the treatments.

3. Results and discussion

3.1. Soil bulk density and water-stable aggregates

Bulk density ranged from 1.13 to 1.24 g cm^{-3} among the treatments (Fig. 2). The GWW treatment (1.13 g cm^{-3}) had the lowest bulk density and it was significantly lower than the CS treatment. The bulk density increased as a function of treatments in the following order: GWW < GB = AG < CS. The measured average bulk density of the buffers was 93% of the CS treatment. Establishment of grass and or agroforestry buffers and grass waterways in row-crop watersheds appeared to significantly reduce ($p < 0.05$) bulk density within five years. Although bulk density was not measured in the 2006 sampling year, other studies conducted on the same watershed support our observations that permanent vegetation reduces soil bulk density (Seobi et al., 2005; Udawatta et al., 2006). Seobi et al. (2005) noticed significantly lower bulk density in the buffer areas than crop areas. Changes in bulk density could be attributed to improvement in soil properties caused by the permanent vegetation. Roots, bio-pores, organic matter, fauna, and other related biological processes, as well as management contributed to improvement in soil physical properties (Seobi et al., 2005; Jiang et al., 2007; Udawatta et al., 2008). The vegetation in the buffers and grass waterways consists of legumes, cool season grasses, and oak trees. Deep-rooted vegetation and the longevity of roots in these areas may have contributed to observed differences in bulk density among the treatments. In addition, buffer and GWW treatments do not experience vehicle traffic, which causes severe compaction when the soil is wet.

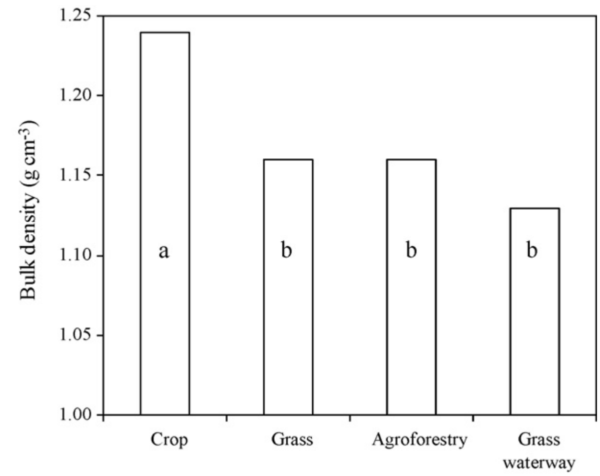

Fig. 2. Soil bulk density for row-crop, grass buffer, agroforestry buffer, and grass waterway treatments. Bars with different letters denote significant differences among treatments at $p < 0.05$.

The crop treatment had the lowest percentage of WSA and the GWW (20.0%) had the highest percentage (Fig. 3). The percentage of WSA in the GWW treatments was significantly greater than the other three treatments. The buffer treatments had significantly more WSA as compared to the CS treatment; two times more than the CS treatment (7.7%). The WSA percentage was not significantly different between the two buffers. The percentage of WSA was significantly different among all three landscape positions. The lower (17.3%) and middle (13.0%) landscape positions contained higher percentages of WSA as compared to the upper landscape position (8.8%; Table 1). The effect of landscape position was significant; WSA increased from the summit to the lower landscape positions.

The distribution of WSA among management practices in this study agrees with previous research (Kremer and Li, 2003; Adesodun et al., 2007; Pikul et al., 2007). For example, Kremer and Li (2003) found a higher percentage (18%) of WSA under various cool season grasses and legumes at sites managed under the "conservation reserve program" as compared to conventionally

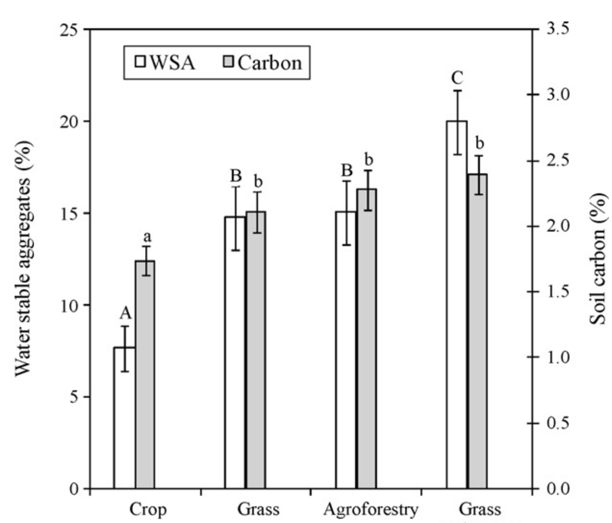

Fig. 3. Water-stable aggregate (WSA) and soil carbon (C) percentages for row-crop, grass buffer, agroforestry buffer, and grass waterway treatments. Bars with upper- and lower-case letters denote significant differences among treatments for WSA and C, respectively, at $p < 0.05$.

Table 1
Percentage of water-stable aggregates by landscape position

Landscape position	Water-stable aggregates (%)
Upper	8.76 ± 1.76
Middle	13.03 ± 1.53
Lower	17.3 ± 1.53

tilled corn (10%) in Missouri. Cultivation reduces WSA irrespective of agroecological region (Adesodun et al., 2007). In this study, trees, several grass species, and the crop as well as agricultural activities influenced aggregate stability.

Improvement in aggregate stability occurs within 2–3 years after establishment of conservation practices (Angers and Carter, 1996; Bissonnette et al., 2001). Results indicate that GWWs with fescue had the most significant effect as opposed to AG and GB treatments. Addition of carbon has been found to be related to increased aggregate stability and increased mean weight diameter (Whalen et al., 2003). However, soil carbon percentages among the two buffers and GWW were not significant (Fig. 3). Although other studies have shown strong relationships between soil carbon and WSA, soil carbon in the current study explained only 31% of the variation in WSA. Soils under fescue grass in the grass waterway may be subjected to additional mechanisms (i.e., high fungal populations involved in aggregation) and more optimum moisture conditions compared with other sites within the watershed. In addition, preferential nutrient enrichment in the larger aggregates occurs in the undisturbed soils as compared to smaller aggregates in the cultivated soils (Adesodun et al., 2007). The differences in WSA and other soil physical parameters among management practices and landscape positions could be attributed to organic matter buildup, displacement, and deposition, as well as higher water content at the lower landscape positions which promote more organic matter buildup and improved aggregate stability.

3.2. Soil carbon

Soil carbon content varied among the treatments; row-crop soils were the lowest in C while GWW soils were the highest (Fig. 4). Soil carbon concentrations in the surface soil were significantly higher for GB, AG, and GWW treatments compared with CS. The carbon percentage in soil under row-crop management was less than 2% whereas the average percentage for the other three treatments was 2.3%. The variation within a management system was insignificant between years. The same pattern continued for both years. The 2-year average carbon percentage for the CS was 1.77% while the buffers had 2.19% and GWW had 2.43%. Among the four treatments, the GWW had the highest carbon concentration in both years. However, the difference was not significant among the permanent vegetative practices.

The upland buffer systems sampled in this study show that soil carbon concentration did not change after five years. A question arises as to whether the greatest soil carbon increase in the surface soil occurs during the first few years of change from a monoculture row-crop to a grass and or tree buffer system. In a mine restoration study, Machulla et al. (2005) observed significant increase in surface soil carbon during the first year and fairly constant dehydrogenase activity during the first three years.

Alley cropping practices could increase soil carbon sequestration tremendously; 154 million ha of crop land in the U.S. under alley cropping could sequester 73.8 Tg C yr^{-1} (Lal et al., 1999; Garrett and McGraw, 2000). Approximately 9.8% of the watershed area in the study is under grass and agroforestry buffer practices. The surface 10-cm of soil contained 26.3 Mt C ha^{-1} in the buffer area as opposed to 22.3 Mt C ha^{-1} in the crop area. A watershed

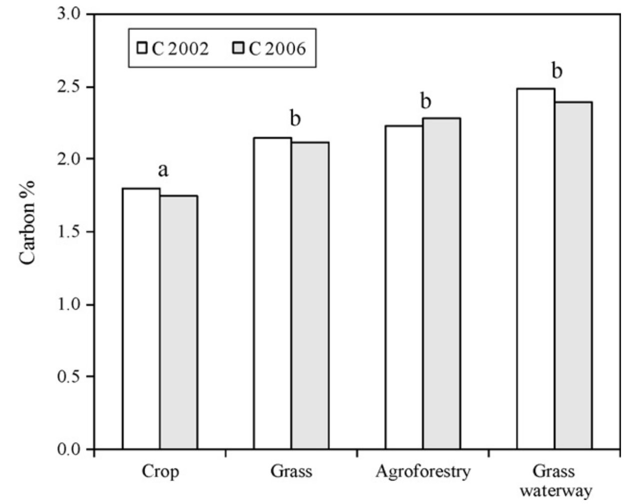

Fig. 4. Total soil carbon percentages for 2002 and 2006 for row-crop, grass buffer, agroforestry buffer, and grass waterway treatments. Bars with different letters denote significant differences among treatments at $p < 0.05$.

with 10% grass and agroforestry buffers would have 22.7 Mt C ha^{-1} in the surface 10-cm of soil, which is about 1.8% more carbon compared with monoculture row-crop management.

Although soil carbon in the buffer areas of this study originated from legumes, grasses, and trees, surface soil carbon did not differ significantly between the two buffer types. Contour buffers similar to this study on row-crop watersheds also receive nutrients in runoff and this synergistic effect may improve further gains. Multispecies and deep-rooted permanent vegetative buffer systems differ widely in quantity and composition of organic materials that are produced, which could support diverse microbial communities and biochemical reactions. Carbon sequestration may also contribute to potential financial and environmental benefits for the landowner.

3.3. Soil enzyme activities

Soil enzyme activities reflect potential rather than determination of actual in situ activity. This is due to the contrasting conditions of the assay relative to the field site, the various enzyme sources and possible confounding chemical reactions that affect the measured activity (Nannipieri et al., 2002). For example, dehydrogenase, considered an intracellular enzyme, may be extracellularly located in soil due to cell lysis and may be associated with organic matter or soil colloidal surfaces (Nannipieri et al., 2002). The above factors must be considered in the interpretation of results and with the assumption that results are relevant in characterizing the functional activity of the soil microbial community present at the date of sampling.

β-Glucosidase enzyme activity (129 μg *p*-nitrophenol released g^{-1} dry soil h^{-1}) was significantly lower in the CS area compared to GB, AG, and GWW treatment areas (Fig. 5A). Among the four treatments, the GB area had the highest level of enzyme activity with 228 μg *p*-nitrophenol released g^{-1} dry soil h^{-1}. The difference between the GB, agroforestry (204 μg *p*-nitrophenol released g^{-1} dry soil h^{-1}), and grass waterway treatments (199 μg *p*-nitrophenol released g^{-1} dry soil h^{-1}) was not significant. On average, permanent vegetative buffers and waterways contained 1.6 times greater enzyme activity than the row-crop treatment.

Similar to β-glucosidase enzyme activity, the activity of glucosaminadase was significantly different between the row-crop treatment and the permanent vegetative treatments (Fig. 5B).

The CS area (73 μg p-nitrophenol released g^{-1} dry soil h^{-1}) had the lowest activity. Grass areas (GB and GWW) exhibited the highest levels of glucosaminidase activity: GWW and GB activities were 135 and 134 μg p-nitrophenol released g^{-1} dry soil h^{-1}, respectively. However, the difference between the grass areas and agroforestry buffer was not significant.

The grassed areas exhibited significantly greater dehydrogenase activity compared with tree + grass vegetation treatments (Fig. 5C). Grass buffers and grassed waterways produced an average of 79 and 65 μg TPF g^{-1} dry soil h^{-1}, respectively. The lowest level of dehydrogenase activity within the cropped area was slightly lower than the AG treatment. The AG and CS treatments showed similar rates of TPF production of 51 and 48 μg TPF g^{-1} dry soil h^{-1}, respectively.

The FDA activity was lowest in the CS area (8 μg fluorescein g^{-1} dry soil h^{-1}) and highest in the GB area (14 μg fluorescein g^{-1} dry soil h^{-1}; Fig. 5D), and differed significantly between CS areas and the other three treatments. The AG and GWW treatments showed similar activities releasing an average of 12 and 11 μg fluorescein g^{-1} dry soil h^{-1}, respectively.

The enzyme activities in this study support the hypothesis that permanent vegetative cover provides favorable conditions for a balanced soil functional diversity reflected by higher enzyme activities compared with soils under row-crop management. Research also shows that no-till land management had higher enzyme activity than conventionally tilled or frequently disturbed soils (Dick et al., 1996; Bergstrom et al., 1998). Variations in tillage system, residue management practice, and cropping practice, as well as the vegetation management influence microbial populations and enzyme activity (Kirchner et al., 1993; Doran et al., 1998; Mungai et al., 2005; Allison et al., 2005). The three permanent vegetative treatments in this study consist of grass, legume, and tree species, thus decomposable organic material, litter quality, and rhizosphere chemistry that influence biochemical characteristics differ among the management practices, which may have contributed to differences in enzyme activities (Lupwayi et al., 1998; Myers et al., 2001; Kremer and Li, 2003; Mungai et al., 2005). In addition, the cessation of both intensive tillage and application of agrochemicals positively affects enzyme activities in the permanent vegetation areas.

Comparisons between X-ray computed tomography-measured pore characteristics (Udawatta et al., 2006) and enzyme activity showed that β-glucosidase, dehydrogenase, and glucosaminadase enzyme activity were positively correlated with the number of pores, porosity and macroporosity. β-Glucosidase showed the best relationship with 0.59 and 0.32 correlation coefficients with the number of pores and soil porosity, respectively. However, FDA activity indicated negative correlations with those two soil pore parameters. Increased enzyme activities in these studies are attributed to increased organic matter and litter quality and

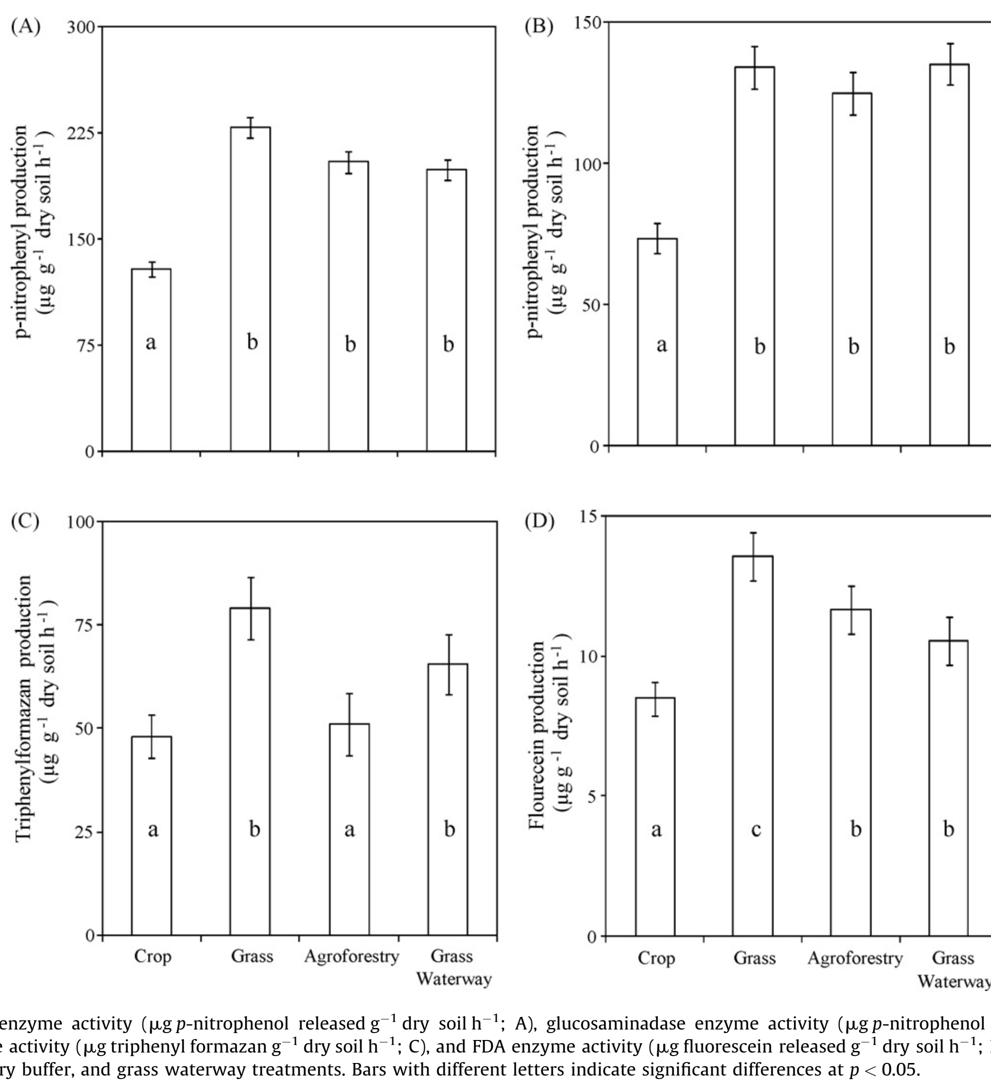

Fig. 5. β-Glucosidase enzyme activity (μg p-nitrophenol released g^{-1} dry soil h^{-1}; A), glucosaminadase enzyme activity (μg p-nitrophenol released g^{-1} soil h^{-1}; B), dehydrogenase enzyme activity (μg triphenyl formazan g^{-1} dry soil h^{-1}; C), and FDA enzyme activity (μg fluorescein released g^{-1} dry soil h^{-1}; D), and for the row-crop, grass buffer, agroforestry buffer, and grass waterway treatments. Bars with different letters indicate significant differences at $p < 0.05$.

quantity as well as improved soil physical parameters. Increased enzyme activity is proportionally linked to microbial function (Caldwell, 2005) leading to improved nutrient cycling and availability, which favors root growth, promotes beneficial plant–microbial interactions, and eventually increases the total soil carbon pool.

The nature of enzymes includes very low mobility in soils. Thus, for the enzymes to have greatest effect, substrates must be near the point of origin of the enzymes. In this upland buffer experimental watershed, buffers are on contours and therefore, runoff water must go though the buffers to exit the watershed. It could be assumed that certain agrochemicals in runoff may be degraded by the diverse microbial communities in these grass and tree buffers. As multispecies buffers support diverse microbial communities, establishment of multispecies buffers may be more environmentally beneficial than a single species buffer.

4. Conclusions

Although the surface soil is physically, chemically, and biologically more heterogeneous compared to subsurface soils, this study confirms that establishment of tree + grass and grass buffers, and grass waterways enhance the soil heterogeneity compared with soils in monoculture row-crop management. Measured physical, chemical, and biological properties show that continuous disturbance has significantly reduced soil quality in the crop areas and the functional diversity of enzyme activity. Establishment of agroforestry buffers on previously cultivated agricultural areas had a significant effect on the measured soil quality indicators in less than 10 years. However, the response varied with vegetation type. Bulk density, aggregate stability, and enzyme activities were greatly improved in the grass waterways with warm season grasses. The vegetation in GWW is much older compared with the grass and agroforestry buffer management sites. Observed physical, chemical, and biological improvements and other associated changes due to establishment of buffers may help reduce nonpoint source pollution from row-crop agricultural lands. Further studies are needed to understand temporal variations and to quantify the influence of buffer age on these parameters. These studies may help identify a unique indicator or combination of indicators to assess soil quality and environmental benefits of conservation buffers.

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